material using a generalized process of some or all of the following steps: 1) clarification, 2) digest of unwanted or contaminating nucleic acid (e.g., Benzonase® treatment), 3) column chromatography (e.g., ion exchange), 4) buffer exchange/concentration (e.g., ultrafiltration) and further nucleic acid removal (e.g., second Benzonase® treatment), 5) polishing (e.g., size exclusion) and 6) sterile filtration.

[0014] Despite decades of refinement, transient transfection has inherent drawbacks. The cost of transfection agents/plasmids, and/or process agents are high and, coupled with the labour-intensive nature of the transfection technique, this makes transient transfection an expensive and technically complex process for clinical/commercial vector production.

[0015] Thus, there is a desire in the art to provide alternative methods of producing viral vectors which help to address the known issues associated with the transient transfection process.

[0016] There has been an attempt to generate stable packaging cell lines in recent years, where viral packaging genes are introduced into eukaryotic host cells along with selection markers such that these genes can be stably integrated into the cell. Similarly, stable producer cell lines also exist where the retroviral genome is also stably integrated. Both of these allow circumvention of a significant portion of the transient transfection process.

[0017] Reference is also made to co-pending EP 17210359.0 application number entitled RETROVIRAL VECTOR, incorporated by reference in its entirety herein, and which describes viral vector production systems and methods employing modular constructs comprising at least two of the nucleic acid components necessary for viral vector production. Such modular constructs were found to provide levels of vector production comparable to those with a traditional multi-plasmid transient process, but allowing for a significant reduction in, for example, the use of transfection agents.

[0018] Thus, there is a desire in the vector manufacturing sector to improve both transient vector production processes as well as the processes for generating stable packaging and producer cell lines.

[0019] The removal of nucleic acids, derived from either production cells or viral vector components, from the final drug product is an important aspect of safety and an area of viral vector manufacturing ripe for improvement. When transformed eukaryotic cell lines, such as HEK293T cells (which also contain Adenovirus E1 and SV40 T antigen genes) are used for production, these cells typically harbour genes with (proto)-oncogenic properties. The inevitable cell death and release of production cell DNA during viral vector manufacture leads to the presence of such (partial and contaminating) sequences within crude harvest material. It is therefore desirable to minimize general DNA contamination (e.g., longer contaminating dsDNA is degraded to short forms within the final product) to preclude the potential for unnecessary & potentially harmful functional gene sequences from being integrated into patient cells during vector delivery. In addition, typical viral vector production methods that transiently transfect production cells with large quantities of plasmid DNA (pDNA) encoding the viral vector components will result in the majority of the contaminating DNA being of vector component origin. As such, it is also desirable to remove contaminating, residual pDNA that could otherwise be taken up and expressed by patient cells.

[0020] From lab scale to industrial scale, viral vector manufacturers have turned to the use of recombinant nucleases, such as derived from *Serratia marcescens* (e.g., Benzonase®) or other commercial hydrolytic nucleases, to treat crude vector material and remove contaminating nucleic acid during the upstream and downstream purification processes. For nuclease treatment during upstream processing, a recombinant nuclease in protein form is typically added to harvest material followed by incubation at less than or equal to 37° for a limited period of time. This, however, represents a potentially avoidable additional processing step, and one in which elevated temperature and increased incubation time may lead to loss in vector stability. To avoid this as a stand-alone step after harvest, a recombinant nuclease is often added into the production cell culture at latter stages of vector production. However, if the half-life of the nuclease is relatively short or activity is insufficient to degrade the required amount of residual nucleic acid, a large amount of nuclease may need to be added at once or continually during these latter stages of culturing. Unfortunately, the use of commercially available recombinant nuclease at this stage of the process often becomes practically burdensome and cost-prohibitive, particularly as scale is increased to hundreds or thousands of litres.

[0021] Thus, there is a desire to improve viral vector production and manufacturing processes so as to streamline and make more efficient the critical step(s) of degrading residual nucleic acid during viral vector production.

SUMMARY OF THE INVENTION

[0022] The invention disclosed herein describes highly effective and streamlined viral vector production systems and manufacturing processes employing the expression and secretion of a hydrolytic nuclease(s) in viral vector production cells during the production of viral vectors. As such, secretion of a nuclease degrades unwanted or contaminating (residual) nucleic acid during viral vector production. In such vector production systems & processes, the nuclease, encoded by a nucleotide expression cassette, is either transiently co-transfected with the viral vector component expression cassette(s) or stably integrated within a production cell genome or nuclease helper cell genome, or nuclease helper cells may be generated by transient transfection with the nuclease expression cassette.

[0023] Due to the complexity of the viral vector production process, expression and secretion of a nuclease from a gene expression cassette in conjunction with a viral vector production system and/or during the viral vector production process is contrary to the state of the art which demonstrates only the expression/co-expression of nuclease in bacterial cells and which, in a viral vector context employs the use of adding commercially available nuclease as a recombinant protein-based enzymatic treatment of viral vector at burdensome upstream and/or downstream time points. It was therefore considered, prior to the invention disclosed herein, that expression and secretion of a nuclease encoded by a nucleic acid expression cassette in conjunction with viral vector component(s) expression cassette(s) in a viral vector production system would result in degradation of necessary vector component DNA thus leading to reduced expression of the vector components, low titres of produced viral vector, and/or toxicity to the viral vector production cells.

[0024] However, in an effort to improve viral vector production manufacturing, the inventors provide the inven-